

INVESTIGATION OF THE POTENTIAL INFLUENCE OF  
FISHERY-INDUCED STRESS ON  
DOLPHINS IN THE EASTERN TROPICAL PACIFIC OCEAN:  
RESEARCH PLANNING

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## ABSTRACT

There is concern that fishing methods used in the eastern tropical Pacific (ETP) tuna purse-seine fishery may cause physiological stress to the dolphin species involved. A workshop to discuss potential methods of measuring fishery-induced stress on dolphins in the ETP was held by the Southwest Fisheries Science Center in La Jolla, California on July 9-11, 1997. The primary goal of the workshop was to identify physiological and morphological indices that can be used to reliably indicate the effects of stress in free-ranging dolphins involved in the tuna fishery. Background information presented at the workshop is included in this report as a description of the main characteristics of the tuna-dolphin fishery and the dolphin species involved, and a discussion of what is known about the current status of dolphin stocks. Some logistical considerations regarding sample collection from dolphins involved in the tuna purse-seine fishery are included. We introduce the concept of stress and our ability to identify and measure stress in mammals, and briefly review recent advances in stress research. We also summarize participant presentations on physiological stress and clinical indices of stress in cetaceans and domestic animals. Three types of investigations that may provide data on the physiological stress response in dolphins involved in the tuna purse-seine fishery are outlined. These include: 1) a Necropsy Program to examine tissues from dolphins killed during fishing operations, 2) a Repeated Chase/Capture study designed to analyze blood chemistry of samples collected from live dolphins during a regime of repeated chase and encirclement, and 3) Historical Analysis examining specimens and data previously collected from dolphins involved in the tuna purse-seine fishery.

## INTRODUCTION

The Declaration of Panama, a 1995 agreement negotiated between the United States and eleven other fishing nations, is one of several recent efforts to reduce dolphin mortality in the eastern tropical Pacific Ocean (ETP) tuna purse-seine fishery. The agreement imposes a total mortality limit of 5,000 dolphins per year, and intends that all countries will take steps to eliminate mortality entirely. The International Dolphin Conservation Program (IDCP) Act, a 1997 amendment to the Marine Mammal Protection Act, was created to implement the agreement by allowing the importation of currently embargoed yellowfin tuna into the United States. The law includes provisions that could allow tuna caught by the intentional encirclement of dolphins with a purse-seine net in the ETP to be declared “dolphin safe” if no dolphins are observed to be killed or seriously injured in that set.

There is concern, however, that fishing methods used by the ETP tuna-dolphin purse-seine fishery may cause stress to dolphins, and that such stress may have an adverse impact on reproduction or survival of these animals. As a result, the IDCP Act requires that research, consisting of population abundance surveys (see Gerrodette *et al.*, 1998) and “stress studies,” be conducted by the National Marine Fisheries Service (NMFS) to determine whether the “intentional deployment on, or encirclement of, dolphins by purse-seine nets is having a significant adverse impact on any depleted dolphin stock.” Initial findings, based on preliminary results of the research program, are to be made by March of 1999, with final conclusions and recommendations reported to congress by the year 2002.

To begin planning for this research, a workshop to discuss potential methods of measuring fishery-induced stress on dolphins in the ETP was held by the Southwest Fisheries Science Center (SWFSC) in La Jolla, California on July 9-11, 1997. The main objectives of the workshop were to summarize the current state of research on stress in non-human mammals and to determine the most appropriate techniques to investigate the potential for fishery-induced physiological stress response in ETP dolphins. Our specific goals were to identify physiological and morphological indices that can be practically measured to quantify the effects of stress in these dolphins. Participants in the workshop included veterinarians specializing in marine mammals, as well as experts on stress physiology, energetics, pathology, reproductive endocrinology, and the ETP tuna purse-seine fishery (Appendix 1).

This report summarizes recommendations of the workshop participants and contains preliminary plans for three specific research projects.

## ARRANGEMENTS FOR THE WORKSHOP

Edwards chaired the workshop, which was convened by Edwards and Curry. The agenda appears in Appendix 2, background papers are listed in Appendix 3.

Several of the participants gave presentations during the meeting (see Appendix 2), and ensuing discussions were, in part, facilitated by a document outlining detailed questions relevant to the objectives of the workshop (Appendix 4).

## BACKGROUND

Background information was presented at the workshop to familiarize participants with general characteristics of the ETP purse-seine fishery. Discussion of background material allowed participants to assess the opportunities and constraints inherent to designing an investigation of the potential for fishery-induced stress on the populations involved. The history of the tuna-dolphin fishery and general points regarding fishing methods were briefly reviewed.

The tuna industry has used the association between tuna and dolphins to fish in the eastern tropical Pacific for nearly five decades. The association occurs frequently enough that sightings of these dolphins are used by fishermen as a signal that tuna may be present, and purse-seine nets are often set on dolphin schools as a means of catching tuna (Perrin, 1969). Pantropical spotted, *Stenella attenuata*, spinner, *S. longirostris*, and common, *Delphinus delphis*, dolphins are the species most often found in association with tuna (predominately yellowfin, *Thunnus albacares*; Hammond, 1981; Au and Pitman, 1988). The reason for the bond, and the relative importance of it, for either dolphins or tuna, is not known (Hammond, 1981; but see Au and Pitman, 1986; Au 1991; Edwards, 1992).

### DOLPHINS INVOLVED IN THE ETP TUNA PURSE-SEINE FISHERY

Edwards presented background information on the primary dolphin species and stocks involved in the ETP tuna fishery (see Perrin *et al.*, 1985; Perrin *et al.*, 1991; Dizon, *et al.*, 1994). Of the dolphin species most frequently targeted (pantropical spotted, spinner, and common dolphins) three major stocks are commonly affected. These are the northeastern spotted dolphin, and the eastern and whitebelly spinner dolphin stocks (Smith, 1983; Wade and Gerrodette, 1993; Wade, 1995). The northeastern spotted dolphin is the target of approximately 85% of all sets on dolphins. Spotted dolphins occur mostly in single-species schools, but a significant fraction of schools also include eastern spinner dolphins.

### Estimated Mortality

Historically, levels of dolphin mortality in purse-seine nets were extremely high (Smith, 1983; Lo and Smith, 1986; Wade, 1995). Wade (1995) estimated that 4.9 million dolphins were killed by the purse-seine fishery over a fourteen year period (1959-1972). Mortality decreased gradually because of measures such as the Marine Mammal Protection Act (1972) and use of equipment designed to prevent dolphin entanglement in purse-seine nets (Barham *et al.*, 1977). Despite these changes, dolphin mortality continued to occur at levels that were probably higher than the stocks impacted by the fishery could sustain (Wade, 1995).

Since 1992, dolphin mortality in the fishery has significantly decreased to a few thousand animals per year (Hall and Lennert, 1993, 1994, 1997; Lennert and Hall, 1995, 1996). A number of legislative measures, including US embargoes and the advent of the "dolphin-safe" policy, caused changes in the fishery and mortality was greatly decreased (see Joseph, 1994). Wade (1995) concluded that these low levels of mortality could allow recovery and growth of the impacted stocks. Total observed mortality for 1996 was 2,547 dolphins (Lennert and Hall, 1997) and the preliminary estimate for 1997 is approximately 3,000 dolphins (Inter-American Tropical Tuna Association, IATTC, 1997).

### Status of Dolphin Stocks

Gerrodette provided information on the population status of pantropical spotted and spinner dolphins in the ETP (see Wade and Gerrodette, 1993; Wade, 1993). Wade and Gerrodette (1993) estimated 730,000 northeastern offshore spotted dolphins, 630,000 eastern spinner dolphins, and 1,000,000 whitebelly spinner dolphins in the ETP. Wade (1993, 1994) determined that the offshore spotted and eastern spinner dolphin stocks were depleted as defined by the Marine Mammal Protection Act. Since 1975, the abundances of offshore spotted and eastern spinner dolphin stocks have apparently been relatively stable. Since about 1992, the abundance of these stocks should be increasing because of the low mortality in the purse-seine fishery. Gerrodette noted that the current observed fishery mortality should have a negligible effect in terms of population dynamics. However, Gerrodette cautioned that Wade's (1994; and see Wade, 1998) model estimating population size of these dolphins was based upon perturbation from mortality and population density, but does not estimate possible effects of fishery-induced stress.

### ETP TUNA PURSE-SEINE FISHERY

Edwards reviewed specific elements of the methods involved in tuna-dolphin purse-seining. The fishery targets yellowfin and skipjack (*Katsuwonus pelamis*; 4% of annual catch) tuna. There are three types of sets: 1) dolphin sets, 2) schoolfish sets, and 3) log sets. The yellowfin tuna known to associate with dolphins are typically 2-4 years old and about 80-125 cm total length. Tuna caught in schoolfish and log sets are generally smaller.

### Oceanographic Location and Seasonal Influences

The yellowfin fishery operates along the coast of Central and South America from Baja California to approximately 20°N, and seaward to approximately 150°W, south of Hawaii (Figure 1).

Although there is fishing effort throughout the year, there is significant spatial, seasonal, and interannual variability of oceanographic conditions (Reilly, 1994). Distribution of tuna and dolphins, and amount of tuna catch, create differences in effort among regions within the fishing grounds.

Scott introduced a discussion of the use of life history parameters to assess changes in population status due to fishery exploitation. Chivers and Myrick (1993) examined life history parameters for two exploited spotted dolphin stocks and found no evidence of expected compensatory changes in response to decreases in abundance. Chivers and DeMaster (1994), however, used a larger data set to investigate life history parameters for three dolphin species (*S. attenuata*, *S. longirostris*, and *D. delphis*) in the ETP and found that compensatory mechanisms did appear to be operating in response to changes in population density. Chivers and DeMaster (1994) concluded that life history data can be used as biological indices to distinguish between a population responding to change in abundance due to fishery exploitation, and changes in carrying capacity when relationships between life history parameters and population status are known. Investigation of life history parameters for ETP dolphin stocks may provide information regarding the relative effects of physiological stress and mortality in these populations. Scott suggested that it may be necessary to include estimates of fishing effort to accurately assess the biological

impact on populations involved. It must be noted that in order to detect changes in life history parameters, large sample sizes will be required.

#### Composition of Fleet

During the late 1990's, the purse-seine fleet in the ETP has included approximately 100-120 vessels. About 50% have been Mexican vessels, the remainder have been from a number of other nations (about six US vessels currently participate in the fleet; under US law it is illegal for these vessels to fish on dolphins).

#### Observer Program

In 1972 the NMFS began a tuna/porpoise observer program to monitor US vessels by collecting data on dolphins and their association with the ETP fishery. In 1979, the IATTC initiated a similar observer program for the international fleet. By 1995 the IATTC had assumed full responsibility for placing observers on all US vessels. Observers are trained to record biological data for all phases of the seining operations. Observer data forms are shown in Appendix 5.

#### Characteristics of Fishing Vessels, Operation and Gear

The Purse-Seining Manual (Ben-Yami, 1994), contains detailed information regarding purse-seines, auxiliary gear, and operation. Purse-seines are rectangular nets (approximately 1.6 km long by 200 m deep) constructed of synthetic materials, and can weigh up to several tons. Vessels involved in the tuna purse-seine fishery are generally 50 to 75 meters long and capable of storing 400-1,700 tons of whole, frozen tuna for months. Fish are stored in large tanks of brine, below decks. Most of the more recently built vessels (after 1970) have carrying capacities (tuna catch) of 1,000-1,400 tons. Vessels require a crew of 15 to 24 men.

Two or three diesel generators provide power to start the main engine(s), run the refrigeration system, and operate electricity. Most boats are equipped with sophisticated navigation and communication equipment and short-range fish-finders.

#### Fishing On Dolphins

Edwards described fishing methods beginning with the search. Fishermen use 25-power and/or hand-held binoculars from the bridge or mast lookout, S-band "bird-radar," and X-band radar to detect cues. A helicopter is used to search for dolphin schools and to assess whether or not a school carries sufficient tuna to make a set. When a dolphin school is located, the seiner approaches traveling at approximately 15 knots. Dolphins are reported to react to the vessels from a distance of approximately eight kilometers.

Four to six speedboats (85-140 hp) are used to chase the school, and to separate and herd a portion of the school to be encircled. The helicopter may also be used to herd animals during these operations, which is said to last from 20-40 minutes but can sometimes take more than two hours.

The purse-seine net is set with the aid of a skiff deployed from the net stack at the rear of the vessel. The skiff acts as an anchor for one end of the net as the seiner moves away in a circle, setting the net continuously behind it. Speedboats herd the dolphins into the closing arc of the purse-seine (dolphins sometimes escape by running across the narrow-



ing opening as the net is pulled into a circle, or diving under the sinking net). Dolphins tend to gather in the center of the net as it is pursed at the bottom. Backdown, a procedure for releasing dolphins over the net's corkline, begins after about two-thirds of the net has been hauled aboard. A channel is formed at the far side of the net and the corkline is submerged so that the dolphins can exit. Speedboats may be used to pull the corkline, thus keeping the channel from collapsing. Crewmen enter the water, when necessary, to pull dolphins over the corkline. Encirclement takes approximately 40 minutes, and dolphins may be confined for about an additional hour (longer if unanticipated problems occur).

### Capture Rate

Dividing the number of dolphins in the ETP by the number of sets annually gives a simplistic capture rate of eight captures/animal/year. An analysis considering the fact that tuna fishermen actively search for, and set on, larger schools (and based on the assumption that individual dolphins choose to remain in schools of a particular size), indicates that about 10% of the northeastern spotted dolphin stock may be set on as often as once per week (Perkins and Edwards, 1997). Approximately 30% of the stock may be set on 2-8 times per year, and about 50% of the stock may be set on only once or twice a year. The analysis, however, is complicated by the fact that dolphin herds are not stable entities and may fluctuate in size as often as on a daily basis.

### Behavior

There is some indication that dolphin behavior has changed since the early years of the fishery. Some information<sup>1</sup> suggests that dolphins in areas with high historical fishing effort are more experienced at avoiding chase and encirclement than more naive dolphins in areas where fishing had expanded more recently. Fishery observers have noted that dolphins apparently anticipate backdown because they move to the backdown channel and "line-up" for release.

Dolphins have been observed being hyperactive as well as passive, when confined in purse-seine nets. Several passive behaviors (sleeping, sinking, and rafting) have been described for dolphins encircled in purse-seine nets, and it has been suggested that such behaviors may have resulted from stress (Norris *et al.*, 1978; Coe and Stuntz, 1980). Scott noted that dolphin behavior in the nets appears to have changed over the years, and that some behaviors are not seen as commonly. There is anecdotal information that "sleeping" behavior, on the net bottom, has become rare. Rafting behavior is still often observed, and has been suggested to be a learned behavior for avoiding net-entanglement, and has also been hypothesized to be induced by stress (Coe and Stuntz, 1980; Sevenbergen, 1997). The significance and current prevalence of these behaviors is not fully known.

## PRACTICAL ASPECTS OF SAMPLE COLLECTION

Scott discussed logistical aspects of sampling and experimental opportunities for studying ETP dolphins.

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1. A study regarding "evasive behavior" of spotted dolphins was completed by G. Heckel, Universidad Autonoma Baja California, Baja California, Mexico, and has been summarized in the NMFS Tuna Newsletter, November 1997.

## CAPTIVE DOLPHINS

Scott suggested that oceanaria or research facilities can provide the necessary environment to allow comparisons between experimental and control animals. Consequently, sophisticated methodologies and rapid laboratory analyses might be more feasible in these settings, than for work at sea. Unfortunately, the only available captive animals are most often species other than those most commonly involved in the fishery (bottlenose dolphins, *Tursiops truncatus*; occasionally live-stranded Atlantic spotted dolphins, *S. frontalis*).

At the present time, there is one female pantropical spotted dolphin at Gulfarium, Florida (captive since 1993), and one male Atlantic spotted dolphin at the Dolphin Research Center, Florida (captive since 1986).

## POST-MORTEM SPECIMENS

There are many samples available from dolphins that have been killed in the fishery over the past twenty-five years. The SWFSC has a large collection of teeth and reproductive organs, as well as skin and blubber tissues (Table 1). In addition, fetuses, stomach contents, adrenal glands, and other tissues have been collected.

Scott noted that observers could begin collecting samples, or whole carcasses, if specimens are required from current fishery kills. Approximately 2,500 – 3,000 animals are now killed per year (700 northeastern spotted dolphins). Not all of these will be available for study, but potentially several hundred samples per year could be collected. This work would require both domestic and international permits.

## LIVE CAPTURE OF FREE-RANGING DOLPHINS

Free-ranging dolphins have often safely been captured while riding the bow of a ship (using a hoopnet or tailgrab). Capture could allow blood samples, heart rate, ultrasound information, and tagging of individuals prior to release. Scott suggested that a comparison between spotted dolphins in Hawaii and those in the ETP might be useful to investigate physical differences between populations that are set on and those that do not experience that type of fishery interaction.

Two successful spotted dolphin purse-seine live-capture/tagging operations were conducted in 1992 and 1993 to track tuna and dolphins in the ETP (Armstrong, 1993, 1994; IATTC, 1993). Dolphins encircled by purse-seine nets were caught by swimmers just prior to backdown. Investigators were able to capture individuals, place them in a raft, collect blood samples, attach a radio transmitter, and release the animal in 7-12 minutes.

It was also noted that biopsy samples are routinely taken, using retrievable biopsy darts, on NMFS marine mammal research cruises. Such samples may be useful for determining cortisol levels in these dolphins (see Research Planning and Appendix 6), as well as for genetic, fatty acid, and isotope analyses. In the ETP, however, biopsies are not always easily taken because many schools actively avoid ships.

## STRESS IN MAMMALS

Subsequent to discussion of background information, the concept of stress and our ability to identify and measure stress in animals was introduced. Animal stress is difficult to

identify and measure, in part, because there is no single consistent biological response to stress (Levine, 1985; Moberg, 1987; Sapolsky, 1994). There have been, however, many recent advancements in stress research. Specifically, many studies have contributed to our ability to measure hormonal interactions and responses to stress (Axlerod and Reisine, 1984; Levine, 1985; Breazile, 1988; Chrousos and Gold, 1992), the effects of stress on suppression of immune function (Keller *et al.*, 1991; Mal *et al.*, 1991), and disruption of reproduction (Moberg, 1991; Rivier and Rivest, 1991). Several recent studies have also been able to characterize and quantify the effects of pursuit or chase on animals (Harlow *et al.*, 1992; Bleich *et al.*, 1994; Bateson and Bradshaw, 1997; see Bateson, 1997).

In an effort to provide a clinical means of measuring of stress in animals, Moberg (1987, 1992) suggested that the most reliable indicator may be the prepathologic state resulting from the stress itself. Based in part on Moberg's work, Dierauf (1990b) provided a preliminary list of clinical indices of stress in marine mammals. Many of the conditions noted by Dierauf (1990b), however, were based on evaluations of captive animals.

Three specialists participating in the workshop gave presentations organized to delineate reliable indicators of stress in the free-ranging dolphins involved in the ETP tuna fishery. Cowan's presentation, based on his extensive research of comparative pathology including human, terrestrial and marine mammal studies, discussed the concept of stress and the potential for measuring stress using animal tissue collected during necropsy. Geraci presented information based on his experience studying stress in marine mammals, and emphasized the known clinical indices of stress in cetaceans. Friend presented results of his research on behavioral and physiological stress in domestic animals and discussed ways in which his methodologies could be applied to dolphins.

### THE MEANING, MECHANISMS, AND CONSEQUENCES OF STRESS

Cowan discussed the concept of stress, describing it as the demand for adaptation to a changing environment. He noted that stressors, which can be defined as any perturbation that threatens an organism's internal homeostasis, incur bioenergetic costs to the individual. Cowan emphasized that, in some circumstances, the extent and nature of the stress response can be influenced by the animal's perception of the stressor (i.e. there is a psychological component to stress). In general, modifications of behavior, activation of the autonomic nervous system, and changes in the neuroendocrine system may all be incorporated into the body's response to stressors (see Moberg, 1985, 1987; Figure 2). The stress response results in the mobilization of energy, the disruption of long-term processes (digestion, reproduction, growth, immune response), diminished sensitivity to pain, and a heightening of the senses (Sapolsky, 1994).

The stress response can range from generalized to acute or chronic, and may eventually become exhaustive. The potential effects, at each of these phases, can vary among individuals. Cowan explained that a generalized stress response, resulting in increased levels of cortisol and other corticosteroids, should be evident in structural changes to the adrenal cortex. An acute stress response may be manifested in autonomic effects such as changes in blood pressure and heart rate, and difficulties in refining temperature control (shivering). A chronic stress response (potentially reaching the level of adrenal exhaustion) involves foregoing costly energetic functions to maintaining basic life processes and can result in reproductive failure, abnormal growth, and immune suppression.

Cowan particularly noted that there appear to be morphological differences between the relative sizes of the delphinid adrenal cortex and medulla in comparison to other mammals. The medulla is very large in dolphins, and Cowan suggested this may be a structural adaptation for responding to the physiological demands of the marine environment. Comparing the weight of the adrenal cortex to the medulla could show response differences between these dolphins and other dolphin populations or even other mammals.

Cowan also emphasized that histological examination of tissues from stranded animals and fisheries kills has indicated chronic stress may cause damage to the heart muscle and vasculature. These ischemic injuries may be caused by long-term exposure to high levels of adrenaline, and could provide an index or an indication of fishery-induced stress in ETP dolphins (see Cowan and Walker, 1979; Cowan *et al.*, 1986).

Building upon the information given during Cowan's presentation, a plan was developed for a proposed necropsy sampling program of dolphins killed in the ETP fishery. This program is discussed in the Research Planning section of this report.

### PHYSIOLOGICAL STRESS RESPONSE IN CETACEANS

Geraci reviewed what is known about stress in cetaceans, presenting examples of clinical measures of stress for several species (see Geraci and Medway, 1973; Geraci and St. Aubin, 1979; Thompson and Geraci, 1986; St. Aubin and Geraci, 1988; Dierauf, 1990b; also see Suzuki *et al.*, 1998). Geraci noted that in adapting to a wide range of conditions in the marine environment, cetaceans have developed some possibly unique features in their physiological response to environmental stressors.

As is common for all mammals, the physiological stress response of cetaceans includes secretion of cortisol from the adrenal cortex to increase lipid and carbohydrate metabolism and promote the breakdown of proteins. Cortisol also reduces the inflammatory response. Geraci said that certain biochemical characteristics of the cetacean physiological stress response appear to differ from those of other mammals. For example, cortisol levels in cetaceans responding to stress appear to be modest in comparison to other mammals (Thompson and Geraci, 1986). Aldosterone, which does not characterize the stress response in terrestrial mammals, is released from the adrenal cortex as a response to stress in cetaceans.

Geraci emphasized that blood chemistry can provide clinical indices of chronic exposure to stress in cetaceans. For example, blood sodium concentration, reduced blood eosinophil concentration, and reduced serum iron levels can be reliable indicators of stress. Other clinical features stress, leading to shock, in cetaceans may include hypoperfusion of the muscle, liver, and other abdominal organs, intravascular coagulation, tissue necrosis, and hypoxia.

Geraci commented that rafting observed in netted dolphins may not be a healthy behavior. He mentioned that harp seals, *Phoca groenlandica*, are known to go into catatonia in initial response to a novel stressor. Geraci also noted that bradycardia and apnea appear to be associated with fear in some phocid seals and might be a part of the clinical profile of dolphins affected by the tuna fishery. He speculated that these dolphins may, with time and experience of being chased and captured, accommodate and be able to avoid that type of parasympathetic stress response.

Building upon information given during Geraci's presentation, plans were developed for a proposed sampling program involving repeated chase and capture of dolphins involved in the ETP tuna purse-seine fishery. The program is described in the Research Planning section of this report.

### BEHAVIORAL AND PHYSIOLOGICAL STRESS RESPONSE IN DOMESTIC ANIMALS

Friend presented a brief history of the study of stress and the stress response. He discussed Selye's (1950) theory of a general adaptation syndrome, noting that the mammalian body has a similar set of responses to a wide variety of stressors. Selye emphasized the role of the adrenal cortex in releasing cortisol under stressful conditions. According to Selye's model, after the initial alarm phase, there is a short-term release of cortisol and after a period of "shock," there may be a low level of cortisol release. Beyond these phases of the stress response, an extreme stressor might lead to an adrenal exhaustion phase.

Friend also remarked on the psychological component of stress, saying that it can play an important role in the degree of response and can lead to variation among individuals. In his own research, Friend has found that an animal's perception of the degree of control it has over a situation will significantly affect the stress response (Friend, 1991). In addition, Friend said that because dolphins are herding animals, like horses and cattle, social separation, such as may occur during chase and encirclement, will likely be stressful to them.

In discussing his research (Friend, 1991; Friend *et al.*, 1988, 1996; Lanier *et al.*, 1995), Friend underscored the importance of integrating knowledge obtained from studying terrestrial mammals into studies of dolphins involved in the ETP fishery. Friend discussed several studies in which domestic animals were separated and maintained in a number of ways, and the differences that were seen in physiological responses to separation and confinement. Friend outlined the methods he has used to measure physiological responses to stress in domestic animals including analysis of blood samples for circulating hormone levels (particularly cortisol) and immunological components, examination of adrenal gland function and morphology, examination of thyroid gland function, and monitoring heart rates. Friend concluded that these measures would likely prove useful in dolphins if the practical problems of measurement could be overcome.

### RESEARCH PLANNING

After initial presentation of background material and relevant information on physiological stress in cetaceans, participants focused on discussing potential research projects that could address the question of whether fishery-induced stress may be having a significant adverse impact on dolphins in the ETP. These discussions led to agreement that three types of investigations may provide useful data: 1) a Necropsy Program to examine tissues from dolphins killed during fishing operations, 2) a Repeated Chase/Capture study designed to analyze blood chemistry of samples collected from live dolphins during a regime of repeated chase and encirclement, 3) Historical Analysis examining specimens and data previously collected from dolphins involved in the tuna purse-seine fishery. The proposed programs are described in general below.

## NECROPSY PROGRAM

### Objectives

The necropsy program proposes to evaluate the pathophysiological condition of post-mortem specimens. Examination of dead dolphins will provide the opportunity to look for morphological indications of physiological stress. Information from post-mortem specimens will also provide insight into the natural states of health and disease for these populations of dolphins in the ETP.

Careful collection and examination of post-mortem specimens will allow histological observation of sublethal effects of fisheries activities on the heart and heart muscle, adrenal glands, the liver and other major organs. The time sequence of injurious events is important and histological evidence of healing, if present, could provide some indication whether observed lesions are several days old or have occurred even more recently.

Relatively fresh blood samples can be obtained from post-mortem specimens to perform complete blood panels, immunological analyses and cell tissue cultures (see Table 2 for some established "normal ranges" in selected blood values for bottlenose dolphins). While information from these analyses may be difficult to interpret, they will at least provide some baseline information for the population.

It is possible that recent estimates regarding frequency of purse-seine capture per individual dolphin (Perkins and Edwards, 1997) can be incorporated into data analyses to arrive at a better understanding of potential effects of capture on the population. Estimating the ages of individuals (Myrick *et al.*, 1983) will be useful for this purpose as well as for use as general life history data.

### Sample Collection

The types of samples proposed for collection are indicated in Table 3. Specimens will have to be collected in a rigorous and systematic manner that is not possible for tuna vessel observers under current circumstances (although certain observer-collected specimens may be used to augment specimens collected for the necropsy program). It will be necessary to have trained technicians or graduate students with some background, or program of study, in pathology to collect samples from specimens. To optimize the amount of information gathered from any single animal, multiple samples must be collected and the procedures could take many hours for a single postmortem specimen.

Cowan emphasized that weighing all organs provides valuable information. In particular the adrenal gland weight could be very important. Microanatomical examination of all organs is essential, and as noted previously, the heart in particular may provide critical information.

Jensen noted the importance of having baseline information on the natural states of health and disease in these dolphin populations. Collected specimens should also be used to provide an overall health assessment. Such information is necessary for deriving conclusions regarding the effects of stress on the animals. Worthy agreed and added that data on body composition and blubber depth will be useful for assessing the general health and nutritional condition of these dolphins.

Czekala added that examination of gonadal tissues can provide basic life history and health information, as well as, indications of compensation for stress.

Palmer presented a method for detecting corticosteroid levels in cetacean blubber samples (Appendix 6) as a means of investigating the stress response in these animals. Geraci indicated that it would be best to obtain baseline information on all steroids in cetacean blubber. Friend agreed and suggested that it would be interesting to look at how all the steroids interact simultaneously. Because we do not know the dynamics of steroids in the blubber, Cowan suggested that it would be useful to assess the metabolic turnover rate by using an exogenous (labeled) steroid in a captive dolphin and measuring diffusion time. Worthy noted that it will also be necessary to know what blubber layer lipids have been extracted from, and the anatomical position from which blubber was sampled.

Curry commented that the chase may be a significant part of the stress on these dolphins, and that the length of the chase may be a factor in the stress response. Geraci noted that there may also be a psychological aspect of the chase affecting the stress response, and that overheated, over exerted muscle does not heal well. Muscle fiber typing, testing for lactic acid and creatine kinase levels, and measuring blood gases may be important for assessment of the degree and effects of muscle and respiratory exertion.

A detailed planning report containing information on major courses of investigation proposed to quantify the effects of stress in dolphins, necropsy procedures, sampling protocols, and strategies for accommodating logistical constraints will be available as the necropsy research is implemented.

## REPEATED CHASE/CAPTURE STUDY

### Objectives

The repeated chase/capture study proposes to provide an accurate documentation of the physiological response to incremental levels of speedboat chase and purse-seine capture using analysis of blood constituents for samples collected from live dolphins that have been subjected to these events. A series of measurements of complete blood panels from a subset of captured dolphins can be used to quantify the effects of repeated chase and capture, and may give an indication of the trend of responses among a number of individuals with repeated exposure over time. In addition, repeated sampling will provide a profile of the range of possible conditions among these animals.

The idea that dolphins could be captured and maintained in a large sea-pen was introduced. This type of temporary captivity would allow the collection and analysis of blood samples to examine changes in blood constituents over time, but would not mimic the conditions of repeated chase and capture. In addition, blood sampling of dolphins in the purse-seine net will provide an opportunity to collect blood from both rafting and non-rafting dolphins.

Purse-seine capture will make it possible to study ETP dolphins while they are being affected, and to examine short-term responses. General protocols will be developed such that dolphins encircled in a tuna purse-seine net will be held by swimmers, placed in a raft for sample collection, roto-tagged and some will be fitted with a radio-transmitter. Handling would most likely occur just prior to backdown (after having been chased for approximately 20 minutes and having spent about one hour encircled by the net). The dolphins would then be released and followed (with the aid of radio-transmitters), and recaptured after a period of one or more days.

Geraci noted that blood analysis is advantageous because biochemical changes to the body almost always precede morphological changes. He gave the example that a blood screen for creatine kinase might indicate an elevated level as evidence of some muscle leakage and possibly muscle damage. Blood samples can also be used to indicate the range of organs affected (see Jain, 1993). In addition, a fresh blood sample can provide immunological data. Czekala suggested that blood samples will be useful for assessing female reproductive status. Blood samples can be used to detect pregnancy, and potentially could show effects of chase on hormone levels of pregnant females (or could provide evidence of abortion during chase or encirclement).

During discussions of the potential for a chase and capture operation, Geraci tabulated predicted changes that might occur in some blood values for dolphins repeatedly captured over time (Table 4).

Geraci also stated that there is enough existing data on normal ranges of baseline cetacean blood values, for reasonable comparisons (see Table 2; see also Medway and Geraci, 1964, 1965; Engelhardt, 1979; Geraci and St Aubin, 1979; Asper *et al.*, 1990; Bossart and Dierauf, 1990; Suzuki *et al.*, 1998).

### Sample Collection

Upon initial capture, a blood sample will be collected and the individual will be roto-tagged for identification. A sub-set of individuals will be fitted with a radio-transmitter so that movement and diving patterns can be monitored. Blood samples (approximately 50 cc) will be collected for each of the roto-tagged dolphins, as is possible. Blood samples can quickly and easily be collected by experienced personnel. Dolphins will be released and, as possible, recaptured up to four times. Sampling and tagging is anticipated to take approximately 10 minutes per individual.

*Terminal Sampling.* One difficulty with designing experimental procedures to examine the physiological stress response in ETP dolphin species, is the lack of a satisfactory control group for comparison of potentially stressed and non-stressed individuals. There is not an unaffected, control population for comparisons. During the workshop, several participants agreed that terminal sampling of individuals during different stages of a repeated chase and capture study would provide controls for making definite conclusions regarding the effects of the purse-seine operations. Although intentionally killing individual dolphins would likely provide definitive control samples, it was not considered to be an acceptable option and was not be considered further.

*Blood.* Collection of blood samples will allow analysis of complete panels (including standard blood chemistry, hormonal measurements, hematological parameters) for all animals possible (see Table 2). In addition, current cell culture techniques may allow useful measurements of immune function and disease resistance. If possible, blood gases will also be measured as an indication of the effects, if any, of forced exercise.

*Other Potential Samples and Measurements.* Workshop participants suggested additional measurements that may provide useful data regarding the effects of chase and encirclement on dolphins. These included monitoring heart rate and completing electrocardiograms for assessment of general cardiac response to chase, capture, and handling. Worthy



noted that assessment of body temperature such as rectal temperature gradient and blubber/muscle temperature gradients would be useful means of examining responses to capture activities.

As measures of general health status, several samples and measurements may be feasible. Culture swabs for microbiological studies (bacteria and viruses) can easily be taken from the blowhole, eyes, genital area, rectum, and any lesions present (Geraci and Lounsbury, 1993; Carter and Cole, 1990). Ultrasonic measurements can be used to measure blubber layer thickness as an index of body condition and to assess pregnancy.

### Logistics

Previous capture operations have provided insight as to the type of vessels, personnel and equipment that will be needed. Extensive pre-cruise practice and testing of research procedures, in addition to prioritizing sample collection, will improve efficiency of the investigation. Only very preliminary logistical requirements are outlined below.

Tagging and capture operations will require the use of a purse-seine vessel so that captures take place under similar conditions to those of the fishery. Use of a research vessel will allow tracking, laboratory work, data analysis, housing of personnel, etc. In addition to transit time, approximately 30 days of ship time during calm seas will be required.

Scientific personnel will include a minimum of the following: One or more veterinarians extensively experienced with the capture, care and handling of marine mammals (veterinary technicians may also be needed), scientists experienced with live-capture operations (particularly with capture by purse-seine of these dolphins in the ETP), scientists experienced with radio/satellite tracking, and marine mammal observers experienced in sighting and identifying dolphins with high-powered binoculars.

Specific types of equipment for animal health assessment (electrocardiograph, ultrasound, etc.) will be required in addition to some shipboard analytical and storage equipment. Underwater video equipment will be used for recording and observations of dolphin behavior in the net.

### ANALYSIS OF HISTORICAL SPECIMENS AND DATA

Workshop participants discussed the scientific value of the numerous specimens collected from dolphins killed in the ETP tuna purse-seine fishery (see Table 1). Many of these samples have already been examined, and have been used to investigate the biology of the populations involved in the fishery (Appendix 7), but these samples may also be valuable for stress-related studies comparing past and present aspects of biology and population status for these animals.

Geraci suggested that the structure of adrenal gland specimens already examined (Myrick and Perkins, 1995) should be reexamined and compared to newly collected specimens.

Any existing blood samples, including those collected by Scott and colleagues (see IATTC, 1993) should also be reexamined by the same laboratory(s) analyzing samples collected in the necropsy program and repeated chase/capture study. Geraci noted that for comparing blood panels, collection (mode of preservation) and laboratory methods must be taken into account. Most clinical laboratories establish their own ranges of normal values, and it is best to have internal standards and use control samples.

## SUMMARY

Extensive discussions of workshop participants led to the development of three types of biological studies to investigate the potential for fisheries-induced stress affecting dolphins in the ETP. These investigations include: 1) a Necropsy Program to examine tissues from dolphins killed during fishing operations, 2) a Repeated Chase/Capture study designed to analyze blood chemistry of samples collected from live dolphins during a regime of repeated chase and encirclement, and 3) Historical Analysis examining adrenal gland, blood, and potentially, life history specimens and data previously collected from dolphins involved in the tuna purse-seine fishery.

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## LITERATURE CITED

- Aguilar, A., and Borrell, A. 1997. Marine Mammals and Pollutants: An annotated bibliography. Fundació pel Desenvolupament Sostenible. 251 pp.
- Armstrong, W. A. 1993. Field notes recorded by NMFS field party leader during the dolphin-safe charter aboard the F/V *Nicole K*: 4 November 1992-7 December 1992. Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, Administrative Report No. LJ-93-16. 95 pp.
- Armstrong, W. A. 1994. Revised and edited field notes 1993 dolphin-tuna tracking study: 6 November 1993-5 December 1993. Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, Administrative Report No. LJ-94-06. 38 pp.
- Asper, E. D., Cornell, L. H., Duffield, D. H., Odell, D. K., Joseph, B. E., Stark, B. L., and Perry, C. A. 1990. Hematology and serum chemistry values in bottlenose dolphins. Pages 479-485 in S. Leatherwood and R. R. Reeves, eds. *The Bottlenose Dolphin*. Academic Press, San Diego.
- Au, D. W. 1991. Polyspecific nature of tuna schools: Shark, dolphin, and seabird associates. *Fishery Bulletin* 89:343-354.
- Au, D. W. K., and Pitman, R. L. 1986. Seabird interactions with dolphins and tuna in the eastern tropical Pacific. *The Condor* 88:304-317.
- Au, D. W. K., and Pitman, R. L. 1988. Seabird relationships with tropical tunas and dolphins. Pages 174-212 in J. Burger, ed. *Seabirds & Other Marine Vertebrates: Competition, predation and other interactions*. Columbia University Press, New York.
- Axlerod, J., and Reisine, T. D. 1984. Stress hormones: Their interaction and regulation. *Science* 224:452-459.
- Barham, E. G., Takaguchi, W. K., and Reilly, S. B. 1977. Porpoise rescue methods in the yellowfin purse seine fishery and the importance of Medina Panel mesh size. *Marine Fisheries Review* 39:1-10.
- Bateson, P. 1997. The behavioural and physiological effects of culling red deer. Report to the Council of the National Trust. 77pp.
- Bateson, P., and Bradshaw, E. L. 1997. Physiological effects of hunting red deer (*Cervus elaphus*). *Proceedings of the Zoological Society of London* 264:1707-1714.
- Ben-Yami, M. 1994. Purse Seining Manual. Fishing News Books, London. 406 pp.
- Bleich, V. C., Bower, R. T., Pauli, A. M., Nicholson, M. C., Anthes, W. A. M. 1994. Mountain sheep *Ovis canadensis* and helicopter surveys: Ramifications for the conservation of large mammals. *Biological Conservation* 70:1-7.
- Bossart, G. D., and Dierauf, L. A. 1990. Marine mammal clinical laboratory medicine. Pages 1-52 in L. A. Dierauf, ed. *CRC Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation*. CRC Press, Boca Raton.
- Breazile, J. E. 1988. The physiology of stress and its relationship to mechanisms of disease and therapeutics. *Veterinary Clinics of North America: Food and Animal Practice* 4:441-480.
- Carter, G. R., and Cole, J. R., Jr. 1990. Diagnostic procedures in veterinary bacteriology

- and mycology. Academic Press, San Diego. 620 pp.
- Cebelin, M. S., and Hirsch, C. S. 1980. Human stress cardiomyopathy. Myocardial lesions in victims of homicidal assaults without internal injuries. *Human Pathology* 11:123-132.
- Chivers, S. J., and Myrick, A. C. 1993. Comparison of age at sexual maturity and other reproductive parameters for the two stocks of spotted dolphin, *Stenella attenuata*. *Fishery Bulletin* 91:611-618.
- Chivers, S. J., and Demaster, D. P. 1994. Evaluation of biological indices for three eastern Pacific dolphin species. *Journal of Wildlife Management* 58:470-478.
- Chrousos, G. P., and Gold, P. W. 1992. The concept of stress and stress system disorders. *Journal of the American Medical Association* 267:1244-1252.
- Close, R. I. 1972. Dynamic properties of mammalian skeletal muscles. *Physiological Reviews* 52:129-197.
- Coe, J. M., and Stuntz, W. E. 1980. Passive behavior by the spotted dolphin, *Stenella attenuata*, in tuna purse seine nets. *Fishery Bulletin*: 78:535-537.
- Cowan, D. F., and Walker, W. A. 1979. Disease factors in *Stenella attenuata* and *Stenella longirostris* taken in the eastern tropical Pacific yellowfin tuna purse seine fishery. Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, Administrative Report No. LJ-79-32C. 21 pp.
- Cowan, D. F., Walker, W. A., and Brownell, R. L. Jr. 1986. Pathology of small cetaceans stranded along southern California beaches. Pages 323-367 in M. M. Bryden and R. J. Harrison, eds. *Research on Dolphins*. Oxford University Press, Oxford.
- Dailey, M. D., and Perrin, W. F. 1973. Helminth parasites of porpoises of the genus *Stenella* in the eastern tropical Pacific, with descriptions of two new species: *Mastigonema stenellae* gen. et sp. n. (Nematoda: Spiruroidea) and *Zalophotrema pacificum* sp. n. (Trematoda: Digenea). *Fishery Bulletin* 71:455-471.
- Dierauf, L. A., ed. 1990a. *CRC Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation*. CRC Press, Boca Raton. 735 pp.
- Dierauf, L. A. 1990b. Stress in marine mammals. Pages 295-301 in L. A. Dierauf, ed. *CRC Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation*. CRC Press, Boca Raton.
- Dizon, A. E., Perrin, W. F., and Akin, P. A. 1994. Stocks of dolphins (*Stenella* spp. and *Delphinus delphis*) in the eastern tropical Pacific: A phylogeographic classification. U.S. Department of Commerce NOAA Technical Report NMFS No. 119. 20pp.
- Edwards, E. F. 1992. Energetics of associated tunas and dolphins in the eastern tropical Pacific Ocean: A basis for the bond. *Fishery Bulletin* 90:678-690.
- Engelhardt, F. R. 1979. Haematology and plasma chemistry of captive pinnipeds and cetaceans. *Aquatic Mammals* 7:11-20.
- Friend, T. H. 1991. Behavioral aspects of stress. *Journal of Dairy Science* 74:292-303.
- Friend, T. H., Taylor, L., Dellmeier, G. R., Knabe, D. A., and Smith, L. A. 1988. Effect of confinement method on physiology and production of gestating gilts. *Journal of Animal Science* 66:2906-2915.

- Friend, T. H., Martin, M. T., Householder, D. H., and Bushong, D. M. 1996. Stress responses of horses during commercial truck transport in hot weather. *Journal of Animal Science* 74:133.
- Gaskin, D. E. 1978. Form and function in the digestive tract and associated organs in Cetacea, with a consideration of metabolic rates and specific energy budgets. *Oceanography and Marine Biology Annual Review* 16:313-345.
- Geraci, J. R., and Medway, W. 1973. Simulated field blood studies in the bottle-nosed dolphin *Tursiops truncatus*. 2. Effects of stress on some hematologic and plasma chemical parameters. *Journal of Wildlife Diseases* 9:29-33.
- Geraci, J. R., and St. Aubin, D. J. 1979. Tissue sources and diagnostic value of circulation enzymes in cetaceans. *Journal of Fisheries Research Board Canada* 36:158-163.
- Geraci, J. R., and Lounsbury, V. J. 1993. *Marine Mammals Ashore: A field guide for strandings*. Texas A&M Sea Grant, Galveston. 305 pp.
- Gerrodette, T., Olson, P., Kinzey, D., Anganuzzi, A., Fiedler, P., and Holland, R. 1997. Report of the survey design meeting for estimating abundance of eastern tropical Pacific dolphins, 1998-2000. December 17-18, 1997. Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, Administrative Report No. LJ-98-03. 25 pp.
- Goforth, H. W. Jr. 1984. Biochemical and anatomical characteristics of dolphin muscles. NOSC Technical Report 940. Naval Oceans Systems Center, San Diego, CA 92152. 41 pp.
- Hall, M. A., and Lennert, C. 1993. Estimates of mortality in the eastern tropical Pacific Ocean tuna fishery in 1991. Report of the International Whaling Commission 43:397-399.
- Hall, M. A., and Lennert, C. 1994. Incidental mortality of dolphins in the eastern Pacific Ocean tuna fishery in 1992. Report of the International Whaling Commission 44:349-351.
- Hall, M.A., and Lennert, C. 1997. Incidental mortality of dolphins in the eastern Pacific Ocean tuna fishery in 1995. Report of the International Whaling Commission 47:641-644.
- Hammond, P. S. 1981. Report on the workshop on tuna dolphin interactions, Managua, Nicaragua. Inter-American Tropical Tuna Commission Special Report 4.
- Harlow, H. J., Lindzey, F. G., Van Sickle, W. D., and Gern, W. A. 1992. Stress response of cougars to nonlethal pursuit by hunters. *Canadian Journal of Zoology* 70:136-139.
- Harvey, S., Phillips, J. G., Rees, A., and Hall, T. R. 1984. Stress and adrenal function. *Journal of Experimental Zoology*. 232:633-645.
- Hohn, A. A., Chivers, S. J., and Barlow, J. 1985. Reproductive maturity and seasonality of male spotted dolphins, *Stenella attenuata*, in the eastern tropical Pacific. *Marine Mammal Science* 1:273-293.
- Howard, E. B. 1983a. *Pathobiology of Marine Mammal Diseases*. Volume I. CRC Press, Boca Raton. 238 pp.

- Howard, E. B. 1983b. Pathobiology of Marine Mammal Diseases. Volume II. CRC Press, Boca Raton. 233 pp.
- Inter-American Tropical Tuna Commission. 1993. Annual Report of the Inter-American Tropical Tuna Commission. Scripps Institution of Oceanography, La Jolla, CA. 316 pp.
- Inter-American Tropical Tuna Commission. 1997. Annual Meeting. October , 1997. La Jolla, CA.
- Jain, N. C. 1993. Essentials of Veterinary Hematology. Lea & Febiger, Philadelphia. 417 pp.
- Jones, T. C., Hunt, R. D., and King, N. W. 1997. Veterinary pathology 6th edn. Williams & Wilkins, Baltimore. 1392 pp.
- Joseph, J. 1994. The tuna-dolphin controversy in the eastern Pacific Ocean: Biological, economic, and political impacts. *Ocean Development and International Law* 25:1-30.
- Junqueira, L. C., and Carneiro, J. 1980. Adrenals, islets of Langerhans, thyroid, parathyroids, and pineal body. Pages 421-443 in L. C. Junqueira, J. Carneiro, and J. A. Long, eds. *Basic Histology*. Lange Medical Publications, California.
- Keller, S. E., Schleifer, S. J., and Demetrikopoulos, M. K. 1991. Stress-induced changes in immune function in animals: Hypothalamo-pituitary-adrenal influences. Pages 771-787 in R. Ader, D. L. Felten, and N. Cohen, eds. *Psychoneuroimmunology*. Academic Press, San Diego.
- Lanier, E. K., Friend, T. H., Bushong, D. M., Knabe, D. A., Champney, T. H., and Lay, D. C. Jr. 1995. Swim habituation as a model for eustress and distress in the pig. 87th Annual Meeting of the American Society of Animal Science, Orlando, Florida, USA, July 25-28, 1995. *Journal of Animal Science* 73: 126.
- Lennert, C., and Hall, M. A. 1995. Estimates of mortality of dolphins in the eastern tropical Pacific Ocean tuna fishery in 1993. Report of the International Whaling Commission 45:387-391.
- Lennert, C., and Hall, M. A. 1996 Estimates of incidental mortality of dolphins in the Eastern Pacific Ocean tuna fishery in 1994. Report of the International Whaling Commission. 46:555-565.
- Lennert, C., and Hall, M. A. 1997. Incidental mortality of dolphins in the eastern Pacific Ocean tuna fishery in 1996. Report of the Scientific Committee of the International Whaling Commission. SC/49/SM1, 12 pp.
- Levine, S. 1985. A definition of stress? Pages 51-69 in G. P. Moberg, ed. *Animal Stress*. American Physiological Society, Bethesda, MD.
- Lo, N. C. H., and Smith, T. D. 1986. Incidental mortality of dolphins in the eastern tropical Pacific, 1959-72. *Fishery Bulletin* 84:27-33.
- Lockyer, C. 1987a. Evaluation of the role of fat reserves in relation to the ecology of north Atlantic fin and sei whales. Pages 183-203 in A. C. Huntley, D. P. Costa, G. A. J. Worthy, and M. A. Castellini, eds. *Approaches to Marine Mammal Energetics*. Society for Marine Mammalogy Special Publication 1.
- Lockyer, C. 1987b. The relationship between body fat, food resource and reproductive

- energy costs in north Atlantic fin whales (*Balaenoptera physalus*). Symposia of the Zoological Society, London 57:343-361.
- Mal, M. E., Friend, T. H., Lay, D. C., Vogelsang, S. G., and Jenkins, O. C. 1991. Behavioral responses of mares to short-term confinement and social isolation. *Applied Animal Behaviour Science* 31:13-24.
- Medway, W., and Geraci, J. R. 1964. Hematology of the bottlenose dolphin (*Tursiops truncatus*). *The American Journal of Physiology* 207:1367-1370.
- Medway, W., and Geraci, J. R. 1965. Blood chemistry of the bottlenose dolphin (*Tursiops truncatus*). *The American Journal of Physiology* 209:169-172.
- Moberg, G. P. 1985. Biological response to stress: Key to assessment of Animal well-being. Pages 27-48 in G. P. Moberg, ed. *Animal Stress*. American Physiological Society, Bethesda, MD.
- Moberg, G. P. 1987. Problems in defining stress and distress in animals. *Journal of the American Veterinary Medical Association* 191:1207-1211.
- Moberg, G. P. 1991. How behavioral stress disrupts the endocrine control of reproduction in domestic animals. *Journal of Dairy Science* 74 : 304-311.
- Moberg, G. P. 1992 Stress induced pathologies in fish: The cost of stress. U.S.Department of Commerce NOAA Technical Report NMFS No. 111:131-134.
- Myrick, A. C., Jr., and Perkins, P. C. 1995. Adrenocortical color darkness and correlates as indicators of continuous acute premortem stress in chased and purse-seine captured male dolphins. *Pathophysiology* 2:191-204.
- Myrick, A. C., Jr., Hohn, A. A., Sloan, P. A., Kimura, M., and Stanley, D. 1983. Estimating age of spotted and spinner dolphins (*Stenella attenuata* and *S. longirostris*) from teeth. U.S. Department of Commerce NOAA Technical Memorandum NMFS No. 30. 17 pp.
- Norris, K. S, Stuntz, W. E., and Rogers, W. 1978. The behavior of porpoises and tuna in the eastern tropical Pacific yellowfin tuna fishery - preliminary studies. *Marine Mammal Commission MMC-76/12*. 86 pp.
- O'Shea, T. J., Brownell, R. L. Jr., Clark, D. R. Jr., Walker, W. A., Gay, M. L., and Lamont, T. G. 1980. Organochlorine pollutants in small cetaceans from the Pacific and South Atlantic oceans, November 1968-June 1976. *Pesticides Monitoring Journal* 14:35-46.
- Perkins, P. C., and Edwards, E. F. 1997. Capture rate as a function of school size in offshore spotted dolphins in the eastern tropical Pacific. Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, Administrative Report No. LJ-97-03. 36pp.
- Perrin, W. F. 1969. Using porpoise to catch tuna. *World Fishing* 18(6):42-45.
- Perrin, W. F., Scott, M. D., Walker, G. J., and Class, V. L. 1985. Review of geographical stocks of tropical dolphins (*Stenella* spp. and *Delphinus delphis*) in the eastern tropical Pacific. U.S. Department of Commerce NOAA Technical Report NMFS No. 28. 28 pp.
- Perrin, W. F., Akin, P. A., and Kashiwada, J. V. 1991. Geographic variation in external morphology of the spinner dolphin *Stenella longirostris* in the eastern Pacific and

- implications for conservation. Fishery Bulletin 89:411-428.
- Reilly, S. B. 1994. Interannual variability of dolphin habitats in the eastern tropical Pacific. I: Research vessel surveys, 1986-1990. Fishery Bulletin 92:434-450.
- Ridgway, S. H., and Patton, G. S. 1971. Dolphin thyroid: Some anatomical findings. *Vergleichende Physiologie* 129-141.
- Rivier, C., and Rivest, S. 1991. Effect of Stress on the activity of the hypothalamic-pituitary-gonadal-axis: Peripheral and central mechanisms. *Biology of Reproduction* 45:523-532.
- Romano, T. A., Ridgway, S. H., and Quaranta, V. 1992. MHC class II molecules and immunoglobulins on peripheral blood lymphocyte of the bottlenosed dolphin, *Tursiops truncatus*. *The Journal of Experimental Zoology* 263:96-104.
- Sapolsky, R. M. 1994. *Why Zebras Don't Get Ulcers*. Freeman, New York. 368 pp.
- Selye, H. 1950. *Stress: A treatise based on the concepts of the general-adaption-syndrome and the diseases of adaptation*. Acta, Inc, Montreal. 822+203 pp.
- Sevenbergen, K. L. 1997. Abnormal behavior in dolphins chased and captured in the ETP tuna fishery: Learned or stress-induced behavior? M. S. thesis, University of California, San Diego. 110 pp.
- Smith, T. D. 1983. Changes in size of three dolphin (*Stenella* spp.) populations in the eastern tropical Pacific. *Fishery Bulletin* 81:1-13.
- St. Aubin, D. J., and Geraci, J. R. 1988. Capture and handling stress suppresses circulation levels of thyroxine (T4) and Triiodothyronine (T3) in Beluga Whales *Delphinapterus leucas*. *Physiological Zoology* 61:170-175.
- St. Aubin, D. J., and Geraci, J. R. 1992. Thyroid hormone balance in beluga whales, *Delphinapterus leucas*: dynamics after capture and influence of thyrotropin. *Canadian Journal Veterinary Research* 56:1-5.
- St. Aubin, D. J., Ridgway, S. H., Wells, R. S., and Rhinehart, H. 1996. Dolphin thyroid and adrenal hormones: Circulation levels in wild and semi-domesticated *Tursiops truncatus*, and influence of sex, age, and season. *Marine Mammal Science* 12:1-13.
- Suzuki, M., Tobayama, T., Katsuma, E., Fusjise, Y., Yoshioka, M., and Aida, K. 1998. Page 131 in *The World Marine Mammal Conference, Abstracts*. Monaco, 20-24 January, 1998.
- Thomson, C. A., and Geraci, J. R. 1986. Cortisol, aldosterone, and leucocytes in the stress response of bottlenose dolphins, *Tursiops truncatus*. *Canadian Journal of Fisheries and Aquatic Sciences* 43:1010-1016.
- Wade, P. R. 1993. Estimation of historical population size of the eastern spinner dolphin (*Stenella longirostris orientalis*). *Fishery Bulletin* 91:775-787.
- Wade, P. R. 1994. Abundance and population dynamics of two eastern Pacific dolphins, *Stenella attenuata* and *Stenella longirostris orientalis*. Ph.D. Dissertation, University of California, San Diego. 255 pp.
- Wade, P. R. 1995. Revised estimates of incidental kill of dolphins (Delphinidae) by the purse-seine tuna fishery in the eastern tropical Pacific, 1959-1972. *Fishery Bulletin* 93:345-354.



- Wade, P. R. 1998. Calculating limits to the allowable human-caused mortality of cetaceans and pinnipeds. *Marine Mammal Science* 14:1-37.
- Wade, P. R., and Gerrodette, T. 1993. Estimates of cetacean abundance and distribution in the eastern tropical Pacific. Report of the International Whaling Commission 43:477-493.
- Walker, W. A., and Cowan, D. F. 1981. Air sinus parasitism and pathology in free-ranging common dolphins (*Delphinus delphis*) in the eastern tropical Pacific. Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, Administrative Report No. LJ-81-23C. 19 pp
- Worthy, G. A. J., and Edwards, E. F. 1990. Morphometric and biochemical factors affecting heat loss in a small temperate cetacean (*Phocoena phocoena*) and a small tropical cetacean (*Stenella attenuata*). *Physiological Zoology* 63:432-442.

**Table 1:** Reproductive organs (and/or reproductive data), teeth, and skin/blubber samples collected in the ETP tuna purse-seine fishery from 1971-1994 and archived at the SWFSC. Columns represent number of individuals. Similar tissue and/or data are available, in significantly fewer numbers, for seven other cetacean species killed in the fishery.

Species or Stock	Reproductive Organs/Data		Teeth	Skin/Blubber
	Males	Females		
Pantropical spotted dolphin	5,903	9,447	18,166	459
Eastern spinner dolphin	1,899	2,027	4,633	92
Whitebelly spinner dolphin	1,892	2,036	4,488	58
Common dolphin	971	1,033	2095	11

**Table 2:** Some published hematological and serum chemistry values for the cetacean species most commonly studied in captivity, the bottlenose dolphin (*Tursiops truncatus*). This information was modified from Bossart and Dierauf (1990) who compiled it in an attempt to provide an indication of normal ranges of blood parameters in cetaceans based on data published by Englehardt (1979) and Asper *et al.* (1990).

Parameter	Unit	Range
<b>Erythrocytes (N = 136)</b>		
Red blood cells	10 <sup>6</sup> /mm <sup>3</sup>	2.9-5.4
Hematicrit	%	36.2-51.0
Hemoglobin	g/dl	12.7-18.1
Mean corpuscular volume	fl	101-143
Mean corpuscular hemoglobin	pg	34-50
Mean corpuscular hemoglobin concentration	g/dl	30-38
<b>Leukocytes (N = 152)</b>		
White blood cells	10 <sup>3</sup> /mm <sup>3</sup>	4.1-10.8
Neutrophils	%	53-62
Lymphocytes	%	15-28
Monocytes	%	0-8
Basophils	%	0-3
Eosinophils	%	7-37
Basophils	%	0-3
Eosinophils	%	7-37
<b>Electrolytes (N = 80)</b>		
Sodium	mEq/l	141-168
Potassium	mEq/l	3.5-5.7
Chloride	mEq/l	100-128
Calcium	mg/dl	7.7-9.7
Phosphorus	mg/dl	4.1-7.8

**Table 2:** Some published hematological and serum chemistry values for the cetacean species most commonly studied in captivity, the bottlenose dolphin (*Tursiops truncatus*). This information was modified from Bossart and Dierauf (1990) who compiled it in an attempt to provide an indication of normal ranges of blood parameters in cetaceans based on data published by Englehardt (1979) and Asper *et al.* (1990).

Parameter	Unit	Range
Iron	ug/dl	110-175 (M) 143-220 (F)
<b>Glucose and lipids (N = 118)</b>		
Glucose	mg/dl	87-150
Cholesterol	mg/dl	87-380
Triglycerides	mg/dl	77-157
<b>Liver, muscle, kidney enzymes (N = 111)</b>		
Creatine kinase	IU/l	14-486
BUN	mg/dl	31-73
Creatinine	mg/dl	1.1-2.5
Uric acid	mg/dl	0.0-1.8
Alkaline phosphatase	IU/l	123-1050
Lactate dehydrogenase	IU/l	159-1393
<b>Liver Enzymes and bilirubin (N = 82)</b>		
Total bilirubin	mg/dl	0.0-0.1
Aspartate aminotransferase	IU/l	48-250
Alanine aminotransferase	IU/l	8-47
<b>Proteins (N = 80)</b>		
Total protein	g/dl	6.2-8.3
Albumin	g/dl	2.8-5.6
Globulin	g/dl	1.1-3.9
<b>Hormones<sup>1</sup> (N = 72)</b>		
Cortisol <sup>2</sup>	ug/dl	8.5-24.2

**Table 2:** Some published hematological and serum chemistry values for the cetacean species most commonly studied in captivity, the bottlenose dolphin (*Tursiops truncatus*). This information was modified from Bossart and Dierauf (1990) who compiled it in an attempt to provide an indication of normal ranges of blood parameters in cetaceans based on data published by Englehardt (1979) and Asper *et al.* (1990).

Parameter	Unit	Range
Aldosterone	pg/ml	3-408
Triiodothyronine (T3), total	ng/dl	81-258
Thyroxine (T4), total	ug/dl	8.5-24.2

1. St. Aubin *et al.*, 1996, measured circulating levels of thyroid and adrenal hormones in 72 free-ranging and captive bottlenose dolphins. They compared hormone levels by sex and status, and found significant differences in the following: cortisol (status), aldosterone (status), total T4 (sex, status), total T3 (sex). The authors concluded that differences in levels of adrenal hormones between wild and semi-domesticated dolphins were indicative of a mild stress response.
2. Suzuki *et al.*, 1998 reported levels of circulating cortisol for 15 cetacean species. Radio Immuno Assay (RIA) was used to measure serum cortisol concentrations for 230 captive bottlenose dolphins (ng/ml,  $11.0 \pm 9.2$ ,  $r=1.8-20.2$ ).

**Table 3a:** A list of some primary modes of investigation and indicators of physiological stress or overall health and disease status for post-mortem specimens of dolphins killed in the tuna purse-seine fishery. The list illustrates types of samples to be collected in the necropsy program. For all animals possible, a complete necropsy will be performed providing detailed descriptions of any grossly evident lesions, weighing all organs, and removing representative tissues from all organ systems. Sample collection will include a variety of preservation methods dependent upon the tissue and analyses required. Selected references are listed in some cases, however Howard (1983a&b), Dierauf (1990a), Geraci and Lounsbury (1993), and Jones *et al.* (1997), have also reviewed relevant aspects of veterinary pathology, cetacean health and disease, and sample collection.

Investigation	Sample	Indicator	Selected References
Liver/pancreatic function	1,2,3	hypoperfusion; elevated blood enzyme levels	Geraci and St.Aubin, 1979
Thyroid gland morphology/function	1,2,3	enlargement; changes in concentrations of T3,T4	Ridgway and Patton,1971; St. Aubin and Geraci, 1988; 1992
Adrenal gland morphology/function	1,2,3	tissue damage; inflammation; enlargement; elevated blood cortisol, aldosterone	Junqueria and Carneiro, 1980; Harvey <i>et al.</i> , 1984
Heart condition	1,2,3	ischemic injuries;myocardial necrosis	Cowan and Walker, 1979; Cowan <i>et al.</i> , 1986; Cebelin and Hirsch, 1980
Muscle condition/function	1,2,3	evidence of myolysis, degradation; fiber typing; pH, lactic acid levels	Close, 1972; Goforth, 1984; Bateson and Bradshaw, 1997

Legend to Tables 3a and b:

- 1 = entire organ/gland/system (as required)
- 2 = organ (tissue) sections
- 3 = blood
- 4 = blubber/skin
- 5 = swabs of body fluids/mucous
- 6 = parasite
- 7 = brain/kidney/liver
- 8 = teeth

**Table 3b:** A list of some primary modes of investigation and indicators of overall health and disease status (and potential indicators of physiological stress) for post-mortem specimens of dolphins killed in the tuna purse-seine fishery.

Investigation	Sample	Selected References
Microbiology/Virology	5	Geraci and Lounsbury, 1993; Carter and Cole, 1990
Reproduction/Life History	1,2,3,8	Hohn <i>et al.</i> , 1985; Chivers and Myrick, 1992; Chivers and Demaster, 1994; Myrick <i>et al.</i> , 1983
Parasitology	1,2,6	Dailey and Perrin, 1973; Walker and Cowan, 1981
Body and nutritional condition	4,2	Lockyer, 1987 a, b; see Worthy and Edwards, 1990
Respiratory condition	1,2	
Gastrointestinal tract condition	1,2	Gaskin, 1978
Kidney condition/function	1,2,3	
Splenic condition	1,2	Cowan and Walker, 1979
Brain condition	1,2	
Toxicology	4,7	Aguilar and Borrell, 1997; O'Shea <i>et al.</i> , 1980
Immunology	2,3	Keller <i>et al.</i> , 1991; Romano <i>et al.</i> , 1992

Legend to Tables 3a and b:

1 = entire organ/gland/system (as required)

2 = organ (tissue) sections

3 = blood

4 = blubber/skin

5 = swabs of body fluids/mucous

6 = parasite

7 = brain/kidney/liver

8 = teeth

**Table 4:** Some predicted changes, over time after repeated chase and recapture, in values from blood and other tissue analyses of ETP dolphins. Changes noted are given in relation to predicted values listed in the previous column. These are theoretical values; actual values would be dependent upon length and intensity of chase, and severity of exertion and psychological affects. These values were presented by Geraci during discussions of the potential for measuring the effects of chase and capture over time. Indications of cumulative effects, if present, were suggested to be most readily apparent on day four.

<b>TIME:</b>	<b>&lt; 6 hours</b>	<b>1 day</b>	<b>2 days<sup>1</sup></b>	<b>4 days</b>
<b>BLOOD LEVELS</b>				
neutrophils	increased	similar values	increased	similar or increasing values
eosinophils	decreased	significantly decreased	decreased	similar or decreasing values
lymphocytes	no effect or slightly decreased	decreased	decreased	similar or decreasing values
iron	no affect or slightly decreased	decreased	similar or decreasing values	similar or decreasing values
cortisol/hydrocortisone	significantly increased; possibly returned to normal	returned to normal	no interaction	no interaction
epinephrine	significantly increased; possibly returned to normal	no interaction	no interaction	no interaction
muscle enzymes	increased	significantly increased	significantly increased	similar or increasing values



**Table 4:** Some predicted changes, over time after repeated chase and recapture, in values from blood and other tissue analyses of ETP dolphins. Changes noted are given in relation to predicted values listed in the previous column. These are theoretical values; actual values would be dependent upon length and intensity of chase, and severity of exertion and psychological affects. These values were presented by Geraci during discussions of the potential for measuring the effects of chase and capture over time. Indications of cumulative effects, if present, were suggested to be most readily apparent on day four.

<b>TIME:</b>	<b>&lt; 6 hours</b>	<b>1 day</b>	<b>2 days<sup>1</sup></b>	<b>4 days</b>
<b>HISTOLOGICAL EVIDENCE</b>				
muscle	no perceptible effect	perceptible effect	perceptible effect	perceptible effect
heart	possible perceptible effect	perceptible effect	perceptible effect	perceptible effect

1. Possible to have no further observed effects on day two. Conditions listed are those that would result to damage to heart muscle or other potential lesions.

## APPENDIX 1

### LIST OF PARTICIPANTS

#### NMFS WORKSHOP TO INVESTIGATE THE POTENTIAL INFLUENCE OF FISHERY-INDUCED STRESS ON ETP DOLPHINS

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## APPENDIX 2

### REVISED AGENDA

#### NMFS WORKSHOP TO INVESTIGATE THE INFLUENCE OF FISHERY-INDUCED STRESS ON ETP DOLPHINS

Southwest Fisheries Science Center  
La Jolla, California

**Thursday, July 10, 1997** (8:30 a.m. - 5:00 p.m.)

##### I. INTRODUCTION

- Who we are, why we are here, what we will do, product anticipated

##### II. INFORMATION PRESENTATION

- ETP Fishery/Dolphin Information (Edwards)
- ETP Dolphin Population Dynamics (Gerrodette)
- Practical Aspects of Sample Collection (Scott)
- Meaning and Measurement of Stress (Cowan)
- Physiological Responses to Stress in Dolphins (Geraci)
- Corticosteroid Detection in Cetacean Blood and Blubber Samples (Palmer)
- Physiological Responses to Stress in Domestic and Other Large Mammals (Friend)

**Friday, July 11, 1997** (8:30 a.m. - 5:00 p.m.)

##### III. QUESTIONS LIST

- Stressors and responses
- Factors affecting stress responses
- Sampling (measuring) stress responses
- Previous/proposed work

##### IV. WORKSHOP SUMMARY

## APPENDIX 3

### SWFSC REPORTS RELATED TO STRESS IN ETP DOLPHINS

- Cowan, D.F. and W.A. Walker. 1979. Disease factors in *Stenella attenuata* and *S. longirostris* taken in the eastern tropical Pacific yellowfin tuna purse-seine fishery. Southwest Fisheries Center Admin. Rept. LJ-79-32C. 20 p.
- Stunz, W.E. and T.B. Shay. 1979. Report on capture stress workshop. Southwest Fisheries Center Admin. Rept. LJ-79-28. 24 p.
- Coe, J.M. and W.E. Stunz. 1980. Passive behavior by the spotted dolphin, *Stenella attenuata*, in tuna purse seine nets. Fish. Bull. 78(2):535-537.
- Myrick, A. 1995. Documentation of progress of stress research projects for future reference. Southwest Fisheries Science Center Admin. Rept. LJ-95-01. 14 p.
- Myrick, A. and P.C. Perkins. 1995. Adrenocortical color darkness and correlates as indicators of continuous acute premortem stress in chased and purse-seine captured male dolphins. Pathophysiology 87. 14 p.
- Perkins, P.C. and E. F. Edwards. 1997. Capture rate as a function of school size in offshore spotted dolphins in the eastern tropical Pacific Ocean. Southwest Fisheries Science Center Admin. Rept. LJ-97-03. 36 p.

## APPENDIX 4

## APPENDIX 5



## APPENDIX 6

### Corticosteroid Detection in Cetacean Blood and Blubber Samples

Jim Palmer  
University of Hawaii at Manoa

Analysis of stress in cetaceans by determining corticosteroid levels in small biopsy samples is conceivable. Corticosteroids are involved in the complex stress response of all vertebrates to both osmotic (mineralocorticoid-mediated) and stress (glucocorticoid-mediated) challenges. Since the evolutionary history of cetaceans includes the unique osmotic challenge of evolution back to the marine environment, and different families of vertebrates utilize different major corticosteroids in the stress response, there was motivation to develop a method which would detect all corticosteroids in a single assay.

The separation of all vertebrate corticosteroids (21-hydroxylated 4-pregnen series): Aldosterone, 18-OH-corticosterone, 18-OH-11-deoxycorticosterone, corticosterone, 11-deoxycorticosterone, cortisone, cortisol, 11-deoxycortisol, and their immediate precursors, 17-hydroxyprogesterone and progesterone by a mode of capillary electrophoresis known as electrokinetic chromatography (EKC) was achieved. This assay is complete in less than twenty minutes and has a biological limit of detection of less than 1 ng/ml using concentrated samples. In addition, the technique separates corticosteroids according to gross structure, allowing the potential for detection of novel corticosteroids

Precise extraction of all corticosteroids from complex sample matrices such as blubber requires a discerning method, as both corticosteroids and potential lipid interferences span a wide range of polarities. A preliminary solvent-extraction gives recoveries between 50-90% for authentic steroids spiked in clean serum samples. The lipid content in Mysticeti samples (blood or blubber) makes them more challenging to extract than Odontoceti. A methanol precipitation is necessary for analysis of high lipid serum, blood lysate, and blubber samples. Recoveries for authentic steroids spiked into blubber samples with the methanol precipitation step are more variable, probably due to compartmentalization of the steroids in the extracted lipids.

Extracted *Tursiops truncatus* serum indicates cortisol was predominant in the animal sampled, with the presence of aldosterone and an unidentified compound with electrophoretic characteristics like 11-deoxycorticosterone and 17-hydroxyprogesterone. *Megaptera novaeangliae*, *Balaena mysticetus*, *Delphinapterus leucas* and other samples are compared. Validation and precise quantification requires optimization of the extraction process and analysis of samples from stressed and unstressed cetaceans. This technique may be developed into a small-volume biopsy (100ul) analysis to provide insight into corticosteroid levels of wild cetaceans.

## APPENDIX 7

### SOME PUBLICATIONS RESULTING FROM BIOLOGICAL DATA COLLECTED FROM DOLPHINS INCIDENTALLY KILLED IN THE TUNA/DOLPHIN PURSE-SEINE FISHERY

- Archer, F. I. 1996. Morphological and genetic variation of striped dolphins (*Stenella coeruleoalba*, Meyen 1833). Ph.D. Dissertation, University of California, San Diego. 185pp.
- Barlow, J. 1984. Reproductive seasonality in pelagic dolphins (*Stenella* spp.): implications for measuring rates. Report of the International Whaling Commission Special Issue 6:191-198.
- Barlow, J. 1985. Variability, trends, and biases in reproductive rates of spotted dolphins, *Stenella attenuata*. Fishery Bulletin 83:657-669.
- Benirschke, K., Johnson, M. L., and Benirschke, R. J. 1980. Is ovulation in dolphins, *Stenella longirostris*, and *Stenella attenuata*, always copulation-induced? Fishery Bulletin 78:507-528.
- Bernard, H. J., and Hohn, A. A. 1989. Differences in feeding habits between pregnant and lactating spotted dolphins (*Stenella attenuata*). Journal of Mammalogy 70:211-215.
- Chivers, S. J. 1992. Life history parameters as indicators of density dependence for populations of delphinids. Ph.D. Dissertation, University of California, Los Angeles. 187 pp.
- Chivers, S. J., and Myrick, A. C. 1993. Comparison of age at sexual maturity and other reproductive parameters for the two stocks of spotted dolphin, *Stenella attenuata*. Fishery Bulletin 91:611-618.
- Chivers, S. J., and Demaster, D. P. 1994. Evaluation of biological indices for three eastern Pacific dolphin species. Journal of Wildlife Management 58:470-478.
- Cowan, D. F., and Walker, W. A. 1979. Disease factors in *Stenella attenuata* and *Stenella longirostris* taken in the eastern tropical Pacific yellowfin tuna purse seine fishery. NOAA, NMFS, Southwest Fisheries Science Center, La Jolla, California. Administrative Report No. LJ-79-32C. 21 pp.
- Dailey, M. D., and Perrin, W. F. 1973. Helminth parasites of porpoises of the genus *Stenella* in the eastern tropical Pacific, with descriptions of two new species: *Mastigonema stenellae* gen. et sp. n. (Nematoda: Spiruroidea) and *Zalophotrema pacificum* sp. n. (Trematoda: Digenea). Fishery Bulletin 71:455-471.
- Dizon, A. E., Southern, S. O., and Perrin, W. F. 1991. Molecular analysis of mtDNA types in exploited populations of spinner dolphins (*Stenella longirostris*). Pages 83-202 in A. R. Hoelzel, ed. Genetic Ecology of Whales and Dolphins. Report of the International Whaling Commission Special Issue 13.
- Douglas, M. E., Schnell, G. D., and Hough, D. J. 1984. Differentiation between inshore and offshore spotted dolphins in the eastern tropical Pacific Ocean. Journal of Mammalogy 65:375-387.
- Edwards, E. F. 1993. Allometry of energetics parameters in spotted dolphin (*Stenella*

- attenuata*) from the eastern tropical Pacific Ocean. Fishery Bulletin 91:428-439.
- Evans, W. E. 1975. Distribution, differentiation of populations, and other aspects of the natural history of *Delphinus delphis* linnaeus in the Northeastern Pacific. PhD Dissertation, University of California, Los Angeles. 147 pp.
- Heyning, J. E., AND W. F. Perrin. 1994. Evidence for two species of common dolphins (genus *Delphinus*) from the North Pacific. Contributions in Science 442:1-35.
- Hohn, A. A., and Hammond, P. S. 1985. Early postnatal growth of the spotted dolphin, *Stenella attenuata*, in the offshore eastern tropical Pacific. Fishery Bulletin 83: 553-566.
- Hohn, A. A, Chivers, S. J., and Barlow, J. 1985. Reproductive maturity and seasonality of male spotted dolphins, *Stenella attenuata*, in the eastern tropical Pacific. Marine Mammal Science 1:273-293.
- Klevezal, G. A., and Myrick, A. C., Jr. 1984. Marks in the tooth dentine of female dolphins (Genus *Stenella*) as an indicator of parturition. Journal of Mammalogy 65:103-110.
- Myrick, A. C., Jr., Hohn, A. A., Sloan, P. A., Kimura, M., and Stanley, D. 1983. Estimating age of spotted and spinner dolphins (*Stenella attenuata* and *S. longirostris*) from teeth. NOAA-Technical Memorandum-NMFS-30, 17 pp.
- Myrick, A. C., Jr., Hohn, A. A., Barlow, J., and Sloan, P. A. 1986. Reproductive biology of female spotted dolphins, *Stenella attenuata*, from the eastern tropical Pacific. Fishery Bulletin 84:247-259.
- Perrin, W. F. 1975. Variation of spotted and spinner porpoise (Genus *Stenella*) in the eastern Pacific and Hawaii. Bulletin of the Scripps Institution of Oceanography 21:1-206.
- Perrin, W. F., and Roberts, E. L. 1972. Organ weights of non-captive porpoise (*Stenella* spp.). Bulletin of the Southern California Academy of Sciences 71:19-32.
- Perrin, W. F, and Henderson, J. R. 1984. Growth and reproductive rates in two populations of spinner dolphins, *Stenella longirostris*, with different histories of exploitation. Report of the International Whaling Commission Special Issue 6:417-430.
- Perrin, W. F., and Reilly, S. B. 1984. Reproductive parameters of dolphins and small whales of the family Delphinidae. Report of the International Whaling Commission Special Issue 6:97-133.
- Perrin, W. F., Warner, R. R., Fiscus, C. H., and Holts, D. B. 1973. Stomach contents of porpoise, *Stenella* spp., and yellowfin tuna, *Thunnus albacares*, in mixed-species aggregations. Fishery Bulletin 71:1077-1092.
- Perrin, W. F., Coe, J. M., and Zweifel, J. R. 1976. Growth and reproduction of the spotted porpoise, *Stenella attenuata*, in the offshore eastern tropical Pacific. Fishery Bulletin 74:229-269.
- Perrin, W. F., Holts, D. B., and Miller, R. B. 1977. Growth and reproduction of the eastern spinner dolphin, a geographic form of *Stenella longirostris* in the eastern tropical Pacific Fishery Bulletin 75:725-750.
- Perrin, W. F., Miller, R. B., and Sloan, P. A. 1977. Reproductive parameters of the

- offshore spotted dolphin, a geographic form of *Stenella attenuata*, in the eastern tropical Pacific, 1973-75. Fishery Bulletin 75:629-633.
- Perrin, W. F., Sloan, P. A., and Henderson, J. R. 1979. Taxonomic status of the southwestern stocks' of spinner dolphin *Stenella longirostris* and spotted dolphin *S. attenuata*. Report of the International Whaling Commission 29:175-184.
- Perrin, W. F., Scott, M. D., Walker, G. J., and Class, V. L. 1985. Review of geographical stocks of tropical dolphins (*Stenella* spp. and *Delphinus delphis*) in the eastern tropical Pacific. NOAA-Technical Memorandum-NMFS-28, 28 pp.
- Perrin, W. F., Akin, P. A., and Kashiwada, J. V. 1991. Geographic variation in external morphology of the spinner dolphin *Stenella longirostris* in the eastern Pacific and implications for conservation. Fishery Bulletin 89:411-428.
- Perrin, W. F., Schnell, G. D., Hough, D. J., Gilpatrick, J. W., Jr., and Kashiwada, J. V. 1994. Reexamination of geographic variation in cranial morphology of the pantropical spotted dolphins, *Stenella attenuata*, in the eastern Pacific. Fishery Bulletin 92:324-346.
- Robertson, K. M., and Chivers, S. J. 1997. Prey occurrence in pantropical spotted dolphins, *Stenella attenuata*, from the eastern tropical Pacific. Fishery Bulletin 95:334-348.
- Rosel, P. E., A. E. Dizon, J. E. Heyning. 1994. Genetic analysis of sympatric morphotypes of common dolphins (genus *Delphinus* ). Marine Biology 119:159-167.
- Schnell, G. D., Douglas, M. E., and Hough, D. J. 1986. Geographical variation in offshore spotted dolphins (*Stenella attenuata*) of the eastern tropical Pacific Ocean. Marine Mammal Science 2:186-213.
- Walker, W. A., and Cowan, D. F. 1981. Air sinus parasitism and pathology in free-ranging common dolphins (*Delphinus delphis*) in the eastern tropical Pacific. NOAA, NMFS, Southwest Fisheries Science Center, La Jolla, California. Administrative Report No. LJ-81-23C. 19 pp.
- Worthy, G. A. J., and Edwards, E. F. 1990. Morphometric and biochemical factors affecting heat loss in a small temperate cetacean (*Phocoena phocoena*) and a small tropical cetacean (*Stenella attenuata*). Physiological Zoology 63:432-442.

In addition, the Report of the International Whaling Commission contains numerous reports on the composition of kill in the ETP tuna purse-seine fishery with length frequencies and life history information (reproductive condition for females of various species). A number of abstracts from the Status of Porpoise Stocks (SOPS) Workshop, 18-22 November 1991 held in La Jolla, CA by the SWFSC, contain biological information from dolphins killed in the fishery.